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REVIEW

Arginine methyltransferases in normal and malignant hematopoiesis

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Arginine methylation is an abundant covalent modification that regulates diverse cellular processes, including transcription, translation, DNA repair, and RNA processing. The enzymes that catalyze these marks are known as the protein arginine methyltransferases (PRMTs), and they can generate asymmetric dimethyl arginine (type I arginine methyltransferases), symmetric dimethylarginine (type II arginine methyltransferases), or monomethyarginine (type III arginine methyltransferases). The PRMTs are capable of modifying diverse substrates, from histone components to specific nuclear and cytoplasmic proteins. Additionally, the PRMTs can orchestrate chromatin remodeling by blocking the docking of other epigenetic modifying enzymes or by recruiting them to specific gene loci. In the hematopoietic system, PRMTs can regulate cell behavior, including the critical balance between stem cell self-renewal and differentiation, in at least two critical ways, via (i) the covalent modification of transcription factors and (ii) the regulation of histone modifications at promoters critical to cell fate determination. Given these important functions, it is not surprising that these processes are altered in hematopoietic malignancies, such as acute myeloid leukemia, where they promote increased self-renewal and impair hematopoietic stem and progenitor cell differentiation.

Recent advances in proteomics and mass spectroscopy have made it possible to study how overall arginine methylation levels change over time as hematopoietic cells differentiate. In one study, primary T-lymphocytes and cell lines were labeled using a methyl-SILAC approach, and the methylated peptides were subjected to mass spectrometry analysis [1]. This analysis identified novel arginine methylated substrates as well as how methylation events change in response to extracellular stimuli (e.g., stimulation of T-cells with CD3, CD28, and interleukin-2). The novel substrates included transcription factors critical for T-cell maturation as well as chromatin-modifying enzymes. These studies clearly indicate that arginine methylation is a dynamically regulated covalent modification in hematopoietic cells. In addition, accumulating evidence suggests that arginine methylation can be a driving force in the initiation and progression of cancer. In this review, we describe in detail those PRMTs with established roles in normal and malignant hematopoiesis: PRMT1, PRMT4, PRMT5, PRMT6, and PRMT7. The known associations between PRMT expression and hematologic malignancies are summarized in Table 1.

PRMT1

PRMT1 is the predominant asymmetric (type I) methyltransferase in mammalian cells, and knockout of *Prmt1* in mice results in embryonic lethality at embryonic day 7.5, with a two-fold reduction in total asymmetric arginine methyltransferase activity [10,11]. Both histone and nonhistone substrates of PRMT1 have been identified in hematopoietic cells. Knockdown of PRMT1 through siRNA-mediated depletion decreased levels of the

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Table 1. Non-histone targets of PRMTs and association with hematologic malignancy

PRMT	Key nonhistone proteins targets	Hematologic malignancies
PRMT1	RUNX1, MLL complex, 53BP1, SAM68, SWI/SNF	AML [2] ALL [3] Lymphoma [4,5]
PRMT4	RUNX1, MLL complex, SWI/SNF, Sm proteins	AML [6] Lymphoma [4,7]
PRMT5	SIN3A/HDAC1, P53, SWI/SNF, E2F, Sm proteins	AML [8] Mantle cell lymphoma [9]
PRMT6	MLL complex, SIN3A/HDAC1, P53	Lymphoma [5]
PRMT7	MLL complex, Sm proteins	Unknown

activating histone mark H4R3me and increased levels of the repressive H3K9me and H3K27me marks at the *β-globin* locus in erythrocytes [12]. The data suggest that PRMT1 promotes an active chromatin state at critical promoters during hematopoietic cell differentiation, as H4R3 methylation by PRMT1 appears to increase the recruitment of the acetyltransferase p300 to histone tails [13]. The ability of the arginine methyltransferases to both directly modify histones and indirectly promote or diminish other histone modifications may explain how changes in PRMT protein expression can have a widespread influence on transcription. The direct and indirect effects of the PRMTs on histone modifications are summarized in Table 2.

PRMT1 can regulate hematopoietic differentiation through the arginine methylation of RUNX1, a transcription factor critical for definitive hematopoiesis, myeloid differentiation, and lymphocyte development (Fig. 1). Methylation of R206 and R210 by PRMT1 on the C-terminus of RUNX1 impairs its association with the SIN3A corepressor, which increases the transcriptional activity of the CD41 promoter, a RUNX1 target that is expressed by primitive multipotent progenitor cells and megakaryocytes [14]. Thus, in this context, PRMT1 appears to regulate transcriptional activation during the normal maturation of the myeloid and erythroid lineages. To determine the biological significance of R206 and R210 methylation, knock-in mouse strains were established, where the arginine residues in RUNX1 were mutated to lysine (from RTAMR to KTAMK) and, thus, were not subject to arginine methylation [15]. Homozygous Runx1$^{KTAMK/KTAMK}$ mice are viable, but exhibit defects in CD3^{+} T-lymphoid cells and CD4^{+} T-cells in the peripheral lymphoid organs. These findings suggest that methylation of RUNX1 by PRMT1 on these sites is critical for proper peripheral T-cell maintenance.

Table 2. Histone modifications and chromatin remodeling by the PRMTs

Arginine methyltransferase	Direct histone modifications	Indirect effects on histone modifications
PRMT1	H4R3	Impaired H3K9me and H3K27me
PRMT4	H3R17 and H3R26	Recruitment of p300 to H3, increased histone acetylation
PRMT5	H2A/H4R3 and H3R8	Impaired H3K9ac
PRMT6	H3R2 and H2AR29	Impaired binding of MLL methyltransferase complex to H3, decreased H3K4me
PRMT7	H4R3 and H3R2	Impaired H3K4me by MLL4

PRMT1 plays important roles in the context of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). PRMT1 is significantly upregulated in newly diagnosed patients with pediatric ALL [3]. The t(8:21) translocation, which occurs in 10% of de novo AML, generates the AML1–ETO fusion protein, which interacts with PRMT1. AML1–ETO has been characterized as both a transcriptional activator and a repressor. Knockdown of PRMT1 reduces the transcriptional activation of target genes activated by AML1–ETO, decreasing proliferation and inhibiting self-renewal [2]. PRMT1 is also found in transcriptional regulatory complexes with mixed lineage leukemia (MLL) fusion proteins, such as MLL-EEN. Together, MLL–EEN and PRMT1 promote H4R3 arginine methylation and expression from the *HoxA9* promoter [16]. Interactions of MLL–EEN with PRMT1, or the PRMT1-interacting protein Sam68, enhance hematopoietic cell self-renewal. Conversely, knockdown of PRMT1 inhibits leukemia transformation. More recently, PRMT1 has been found to be required for leukemia induction by the leukemia fusion proteins MLL–GAS7 and MOZ–TIF2 in mice [17]. Although PRMT1 is overexpressed in Hodgkin's lymphoma (HL) cell lines and primary lymphoid tissue, the significance of PRMT1 in HL pathogenesis is unknown [4].

The regulation of splicing by the PRMTs is another example of a process in which changes in PRMT expression or activity can contribute to the initiation or maintenance of hematologic malignancies. The spliceosome is a multiprotein complex containing small nuclear ribonucleic proteins, and components of this complex are subject to mutations in 50% of patients with myelodysplastic syndrome [18]. Specific RNA-binding proteins are recognized as targets of PRMT1, including RBM15, a protein that recruits the splicing factor SF3B1 to intronic regions of genes that are critical for megakaryocytic development (such as *GATA1* and *RUNX1*) [19]. Methylation of RBM15 (a gene involved in an acute megakaryocytic leukemia-associated translocation) decreases RBM15 protein levels by triggering ubiquitin–proteosome-mediated degradation. Ultimately, this leads to inhibition of megakaryocytic differentiation. In summary, accumulating evidence suggests that small molecule inhibitors of PRMT1 may exhibit efficacy in AML, ALL, and HL.

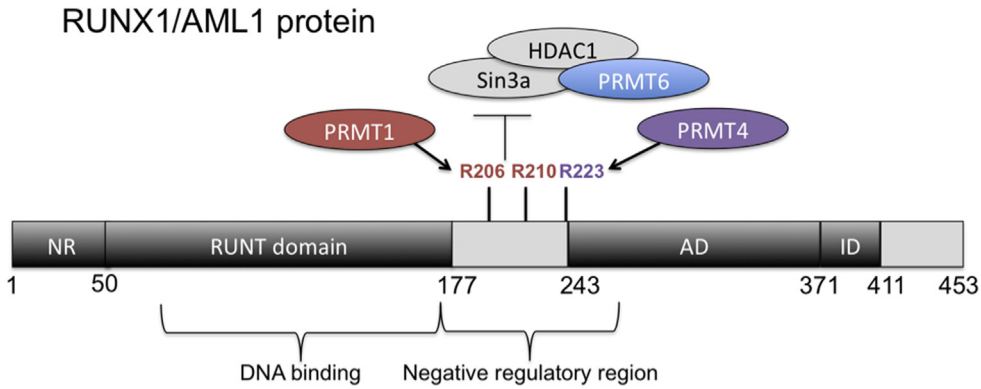

Figure 1. Diagram of the RUNX1 protein and sites of regulation by the PRMT family. The RUNX1 transcription factor is directly modified by PRMT1 on R206 and R210, which prevents its association with the SIN3A co-repressor and promotes its transcriptional activity. PRMT4 can directly modify RUNX1 on R223, which triggers the formation of a repressive complex at RUNX1 target genes, such as mir223. PRMT6 and RUNX1 are also found in a co-repressor complex containing SIN3A and HDAC1 in human $CD34^{+}$ cells; however, this interaction (between RUNX1 and PRMT6) is lost following megakaryocytic differentiation.

PRMT4/CARM1

PRMT4 was first identified as a transcriptional activator; it methylates histone H3 at the unique sites H3R17 and H3R26 and methylates many nonhistone substrates. PRMT4 knockout mice are born, but they die shortly after birth from defects in the differentiation of the lung parenchyma, adipocytes, and muscle cells [20]. T-cell differentiation is also highly dependent on PRMT4 expression, as PRMT null embryos exhibit severe defects in thymocyte progenitor maturation [21]. PRMT4 has also been found to regulate cell fate decisions at the earliest stage of embryogenesis [22]. The blastomere inner cell mass expresses high levels of PRMT4, which positively regulate the expression of key pluripotency genes, including Nanog and Sox2. PRMT4 depletion downregulates these pluripotency genes, leading to embryonic stem cell differentiation [23]. PRMT4 also modifies nonhistone substrates, such as MLL1/2, modulating its binding to specific promoters during differentiation [24]. PRMT4 methylates other transcriptional co-activators such as the steroid receptor co-activator AIB1 [25]. It has also been implicated in the regulation of splicing through the post-translational modification of multiple splicing factors, including CA150, SAP49, SmB, and U1C [26]. In general, methylation of these splicing factors by PRMT4 promotes alternative splicing through exon skipping.

Our lab recently reported that PRMT4 is overexpressed in patient AML samples and that PRMT4 negatively regulates myeloid differentiation [6]. PRMT4 levels are highest in undifferentiated human $CD34^{+}$ cells, with decreased expression as cells undergo cytokine-driven myeloid differentiation in vitro. Furthermore, overexpression of PRMT4 blocks the myeloid differentiation of human HSPCs, whereas its knockdown induces their myeloid differentiation. Our lab identified a feedback loop, whereby PRMT4 modifies RUNX1 on arginine 223, leading to the recruitment of a DPF2-containing repressor complex. Depletion of PRMT4 (or DPF2) results in differentiation of myeloid leukemia cells in vitro and their decreased proliferation in vivo, suggesting that targeting PRMT4 may be an effective therapeutic strategy for AML.

PRMT4 has also been implicated in modulating the activity of the SWI/SNF (BAF) chromatin-remodeling complex, one of the most commonly mutated protein complexes in cancer. SWI/SNF complexes control the transcriptional regulation of genes involved in differentiation and cell proliferation, and BAF155/SMARCC1, a core component of the SWI/SNF complex, is a substrate of PRMT4 [27]. Methylation of BAF155 on R1064 affects the targeting of the SWI/SNF complex and upregulation of the c-Myc pathway. Overexpression of PRMT4 and increased BAF155 methylation were both seen in breast cancer samples obtained from patients with the poorest survival outcomes. The regulation of SWI/SNF complexes by PRMT4 does not appear to be limited to solid tumors. Methylation of the transcription factor CEBPB or RUNX1 by PRMT4 interferes with their binding to components of the SWI/SNF complex [6,28]. In summary, PRMT4 may be an important regulator of SWI/SNF targeting and transcriptional regulation in a variety of cancer cells.

PRMT5

PRMT5 was originally identified as a JAK kinase-binding protein in yeast two-hybrid assays; it was later found to possess methyltransferase activity toward arginine residues [29,30]. We now know that PRMT5 is the major type II enzyme in mammalian cells, catalyzing the symmetric dimethylation of arginine residues in histone (at H2A/H4R3 and H3R8) and numerous nonhistone proteins, including MBD2, p53, HOXA9, and Sm Ribonucleoproteins [31–34]. PRMT5-mediated methylation of histones is generally thought to repress gene transcription; indeed, PRMT5 is found in association with multiple co-repressor complexes, including SIN3A/HDAC and MBD2/NuRD [35]. PRMT5 also regulates RNA splicing by methylating

the core snRNP components, SmB/B′ and SmD, thereby facilitating biogenesis of the spliceosome [36,37]. Loss of PRMT5 has been reported to affect splicing of numerous genes in neural stem/progenitor cells, including MDM4, a negative regulator of p53 [38,39]. In addition to transcription regulation and RNA splicing, PRMT5 regulates many other cellular processes, including signal transduction and cell cycle progression [12,40].

PRMT5 functions in the context of several multimeric complexes, which invariably contain the WD40-repeat protein MEP50, but also specific nuclear and cytoplasmic components [41]. MEP50 is indispensable for PRMT5 enzymatic activity, whereas these other components regulate PRMT5 localization and substrate specificity. In the cytoplasm, the PRMT5/MEP50 core complex interacts with pICln or RioK1, which directs PRMT5 methyltransferase activity toward Sm proteins or nucleolin, respectively [34,42]. In the nucleus, the core PRMT5 complex interacts with COPR5 (coordinator of PRMT5), which promotes the methylation of H4R3 by PRMT5, but also inhibits the ability of PRMT5 to methylate H3 at R8 [43]. The formation of PRMT5 complexes can be regulated by upstream signaling pathways. For example, our lab has previously found that the myeloproliferative neoplasm-associated mutant JAK2 kinase (JAK2V617F) gains the ability to phosphorylate PRMT5 and disrupt the interaction between PRMT5 and MEP50, thus inhibiting PRMT5 methyltransferase activity [44]. PRMT5 function is also regulated by its subcellular localization, with cytoplasmic and nuclear PRMT5 having distinct effects on stem cell development and possibly cancer cell proliferation [45]. How cells control the translocation of PRMT5 between the cytoplasm and the nucleus is poorly understood.

PRMT5 has been reported to play an important role in maintaining the pluripotency of both embryonic and adult stem cells by inhibiting the expression of differentiation-associated genes [46,47]. To study the function of PRMT5 in hematopoietic stem cells (HSCs), our lab generated an inducible PRMT5 conditional knockout mouse. We deleted PRMT5 in the hematopoietic cells of 2-month-old mice and triggered a rapidly fatal bone marrow aplasia. We observed a cell-intrinsic, nonhomeostatic transient expansion of PRMT5-null HSCs, which identified a role for PRMT5 in maintaining stem cell quiescence. However, the exhaustion of both HSCs and hematopoietic progenitor cells (HPCs) in PRMT5-null mice indicated the indispensable role of this methyltransferase in maintaining normal HSPC function. We have completed several mechanistic studies to date that identified severely impaired cytokine signaling and overactive p53 signaling in PRMT5-deficient HSPCs, which may explain some of the phenotypes we observed [48].

Growing evidence suggests that PRMT5 is involved in tumorigenesis. Although recurrent mutations of PRMT5 are rarely observed in cancer cells, its expression level is upregulated in leukemia, lymphoma, and many solid tumors [8,9,49]. PRMT5 is required for tumor cell proliferation, and overexpression of PRMT5 transforms cells [50]. The oncogenic role of PRMT5 in lymphomagenesis has been extensively studied; elevated levels of PRMT5 protein are found in primary lymphoma samples and cell lines, leading to repression of the tumor suppressor RB proteins (RB1, RBL1, and RBL2) [8]. PRMT5 upregulation is induced by the ectopic expression of several oncogenes, including Notch1, MLL-AF9, and Myc, and PRMT5 has been reported to cooperate with mutant cyclin D (D1T286A) to induce lymphomagenesis in mice [51]. Treating mantle cell lymphoma cells with PRMT5-specific inhibitors exhibited promising in vitro and in vivo anti-tumor efficacy [52]. The role of PRMT5 in the myeloid malignancies is still under investigation. Although we have found that loss of PRMT5 in normal HSPCs leads to reduced cell surface expression of FLT3 (Fms-related Tyrosine Kinase 3), perhaps via the aberrant splicing of *Flt3* pre-mRNA, a recent report suggested that PRMT5 inhibition reduces FLT3 levels via effects on mir-29b expression in AML cells [53]. Given the high frequency of FLT3 mutations in AML, PRMT5 could be a potential target for this subset of myeloid malignancies. Several recent reports have also described how deletion of MTAP, a gene that encodes an enzyme critical for methionine metabolism, may sensitize cells to PRMT5 inhibition [54,55]. Because of its proximity to the tumor suppressor gene *CDKN2A* on 9p21, MTAP is deleted in several solid tumor types as well as diffuse B-cell lymphoma. MTAP-deficient cancer cells accumulate a metabolite (MTA) that inhibits PRMT5 activity, thus sensitizing them to further PRMT5 inhibition. These reports suggest that the subgroup of cancer patients with MTAP deletion may be ideal candidates for treatment with PRMT5 inhibitors.

PRMT6

PRMT6 is a type I arginine methyltransferase that is generally thought to methylate histone H3R2, a histone modification that is associated with decreased levels of the transcriptional activation mark H3K4me [56–59]. The global distribution of H3R2me2a was mapped by characterizing the regulatory regions of 200 genes in leukemia and lymphoma cell lines. Interestingly, H3R2me2a was found primarily at inactive promoters. Although H3R2me2a was also localized in gene bodies, this had no correlation with gene expression.

These studies also indicated that the antagonist relationship between the methylation of H3R2 and H3K4 is reciprocal. The presence of H3K4 methylation on H3 peptides prevented the ability of PRMT6 to methylate H3R2. Conversely, the presence of H3R2me2a interfered with recognition of the H3 tail by WDR5, a component of the MLL methyltransferase complex that is necessary for the bridging of the MLL methyltransferase and H3 [56,57].

Thus, PRMT6 may antagonize the action of MLL complexes, preventing the deposition of the H3K4 methylation mark on promoter regions. In support of this hypothesis, overexpression of PRMT6 was found to inhibit the recruitment of the MLL methyltransferase complex to H3K4, and it resulted in the decreased transcription of Hox- and Myc-dependent genes. This process may be critical in regulating embryonic stem cell differentiation and self-renewal, as PRMT6 protein levels and H3R2 methylation levels increase with the differentiation of mouse embryonic stem cells, coinciding with decreased expression of genes regulating pluripotency and self-renewal [59].

PRMT6 can also regulate lineage commitment at later stages of hematopoietic differentiation through the modification of RUNX1 function. Before megakaryocytic differentiation, RUNX1 and PRMT6 are co-localized within a co-repressor complex containing SIN3A and HDAC1, which can be found at the promoters of megakaryocytic genes such as CD41 [60,61]. These genes exhibit increased expression of H3R2me and decreased H3K4me. However, after megakaryocytic differentiation, RUNX1 is found in a co-activator complex that lacks PRMT6 but contains p300 and WDR5. Decreased interaction with PRMT6 appears to enhance the ability of RUNX1 to bind DNA on the regulatory regions of genes such as PU.1. The mechanism that regulates the recruitment or exclusion of PRMT6 at these regulatory regions is still unknown, but may be related to other posttranslational modifications of RUNX1.

Although PRMT6 null mice are viable, mouse embryo fibroblasts from these mice display increased cellular senescence because of high levels of p53 [62]. PRMT6 and H3R2me2a were found to be enriched in an upstream regulatory region that controls p53 expression by chromatin immunoprecipitation. PRMT6 has also been reported to directly repress the cyclin-dependent kinase p21 promoter in breast cancer cells [63]. This suggests that PRMT6-mediated repression of p53 and p21 may be a critical function of this enzyme, and it supports the hypothesis that PRMT6 functions as an oncogene, as PRMT6 overexpression promotes growth and prevents cellular senescence.

PRMT6 is overexpressed in several solid tumor types, including breast, bladder, prostate, lung cancer, and lymphoma. Although PRMT6 overexpression was observed in 40% of lymphoma patients based on cDNA microarrays, PRMT6 overexpression has not been reported in other hematologic malignancies at this time [5]. Whether the oncogenic effects of PRMT6 are driven primarily by its roles in impaired differentiation, increased cell cycle progression, or decreased cellular senescence remains a critical question.

PRMT7

PRMT7 is unique in that it is considered a type III arginine methyltransferase, capable of forming only monomethylarginine [64]. Mice lacking PRMT7 are viable but die 5–10 days after birth because of skeletal abnormalities, lower body mass, and decreased red blood cell counts [65].

PRMT7 has been implicated primarily in regulating the DNA damage response. PRMT7 is associated with sensitivity to DNA-damaging agents, such as topoisomerase inhibitors, whereas another study found that PRMT7 and dimethylated H2AR3 and H4R3 are enriched at DNA repair genes [66–68]. Knockdown of PRMT7 negatively regulates the expression of multiple genes involved in DNA repair, including *ALKBH5*, *APEX2*, *POLD1*, and *POLD2* [69]. This study implicated the catalytic subunit of DNA polymerase (POLD1) as the main mediator of sensitivity to DNA damage. Like PRMT5, PRMT7 methylates the Sm spliceosome proteins and may play a role in mediating snRNP assembly and RNA splicing [70]. Although alternative splicing regulated by PRMT5 has been found to modulate the DNA damage response, the role of PRMT7 in this process has not been as extensively studied. Overall, PRMT7 positively regulated genes in the DNA repair pathway, and PRMT7 overexpression may desensitize cancer cells to DNA-damaging cancer therapies.

Like PRMT6, PRMT7 can antagonize the action of the MLL methyltransferase complex. MLL4 is the major mammalian H3K4 mono- and dimethyltransferase and is critical for cellular differentiation. Increased MLL4-catalyzed H3K4 methylation is associated with a decrease in the PRMT7 methylation mark H4R3me1s. Knockdown of PRMT7 enhanced the levels of H3K4me3, increased the expression of MLL4 target genes, and promoted neuronal differentiation [71]. PRMT7 also appears to be important for B-cell differentiation, as it is highly expressed in lymphoid tissues [72]. Conditional knockout of PRMT7 in the B-cell lineage resulted in impaired B-cell differentiation and hyperplasia of the germinal center, whereas overexpression of PRMT7 triggered an increase in Bcl6 in germinal center-derived B-cell lines. Together, these studies suggest that PRMT7 overexpression impairs lymphoid differentiation and the DNA damage response; however, PRMT7 has not been associated with the hematopoietic malignancies to date.

Conclusions and future directions

In summary, the protein arginine methyltransferases are important regulators of HSPC self-renewal, differentiation, and proliferation. The ability of PRMTs to modify both transcription factors and histone substrates, via assembly into various large multiprotein complexes, allows them to control the activation and repression of the genes that regulate these biological processes. In fact, RUNX1 is a key example of how multiple modifications catalyzed by different PRMT family members can have opposing effects on the same substrate. Like RUNX1, the MLL methyltransferase complex and SWI/SNF chromatin-remodeling complex appear to receive multiple inputs from PRMTs.

Finally, the role of multiple PRMTs in regulating alternative splicing may greatly impact the proliferation, differentiation, and survival of HSPCs. Although these targets are biologically important, it is likely that many additional targets for these enzymes remain to be discovered. Accumulating evidence suggests that the PRMTs are important in the pathogenesis of hematologic malignancies. Because of the importance of AML1–ETO and MLL-fusion proteins in leukemia development, their dependency on PRMTs may be important for their function and could be exploited therapeutically. Inhibitors targeting PRMT1, PRMT4, PRMT5, and PRMT6 are already in development and show great promise as novel therapeutics for the hematologic malignancies.

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Conflict of interest disclosure

The authors have no conflicts of interest to disclose.

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